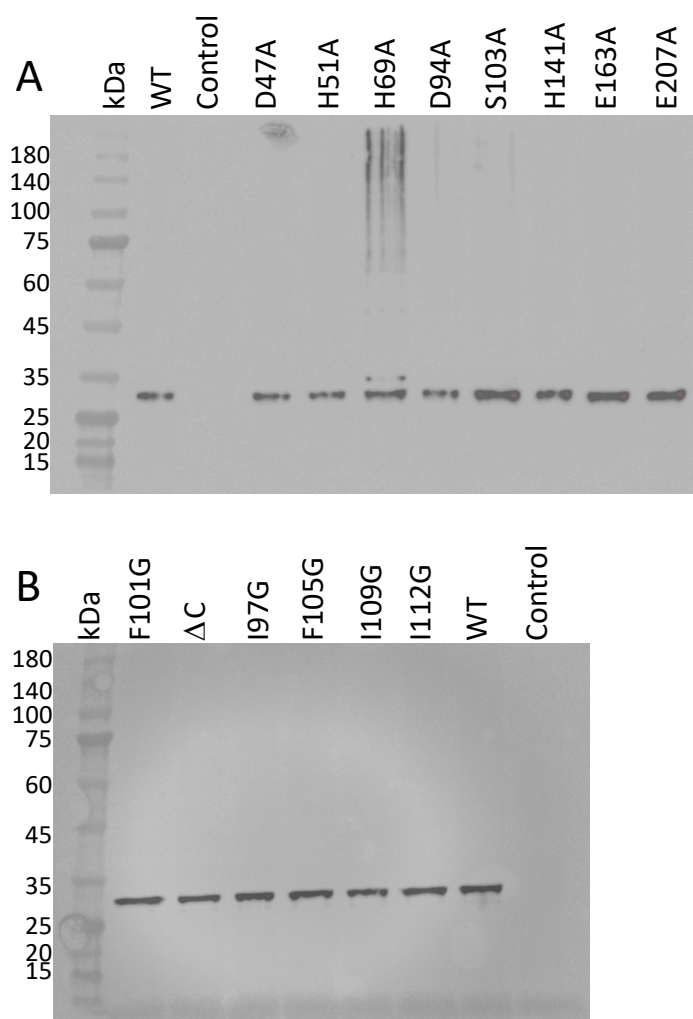
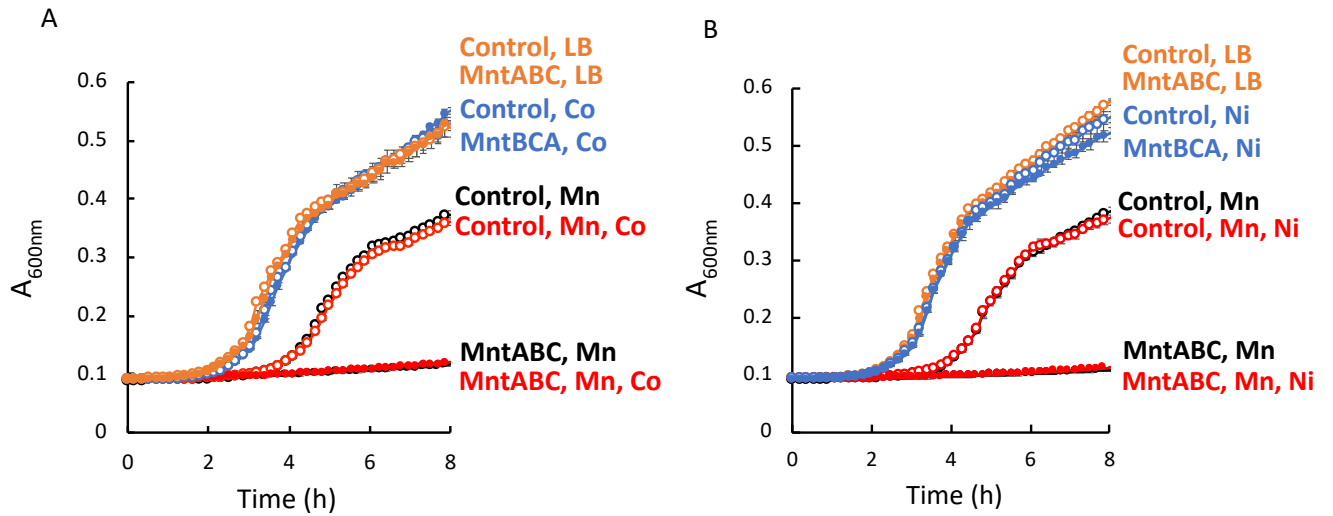


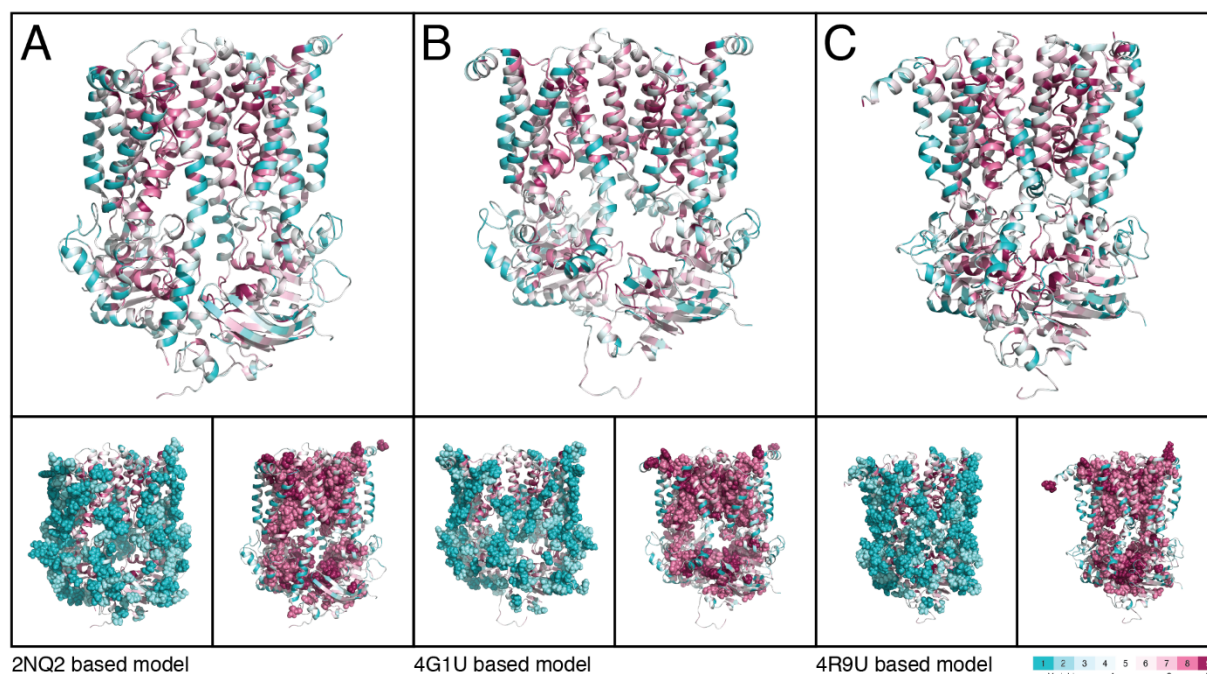
Supplementary Figure 1. Metal accumulation mediated by baMntBCA. Mid exponential phase cultures of *Bacillus subtilis* cells transformed with either a control plasmid (open bars) or a plasmid harboring the complete baMntBCA operon (grey bars) were incubated for 15 min with the indicated concentrations of MnSO₄. Cells were then harvested, washed with PBS-EDTA buffer and their intracellular Mn²⁺ content was determined by ICP-MS. Shown are averages of technical triplicates of experiments conducted at least three times. Error bars represent the standard deviations. * p<0.05; ** p<0.01; *** p<0.001; two-tailed student t-test.



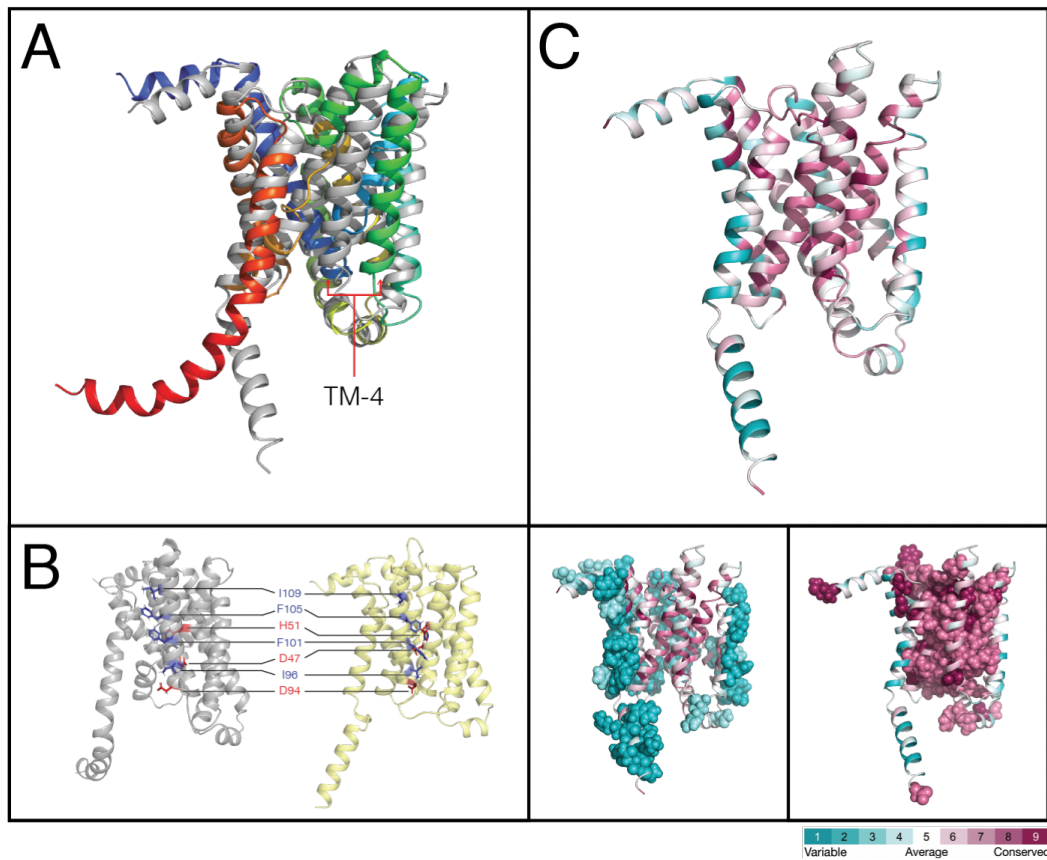
Supplementary Figure 2. Expression of WT and mutant baMntBC-A. Membranes fractions were prepared from cells transformed with an empty integration vector (control), or a plasmid encoding WT _{baMntBCA} or the indicated mutants. 1 μ g of total membrane protein were separated by SDS-PAGE and the protein content was visualized by immuno-blotting using an α -His antibody. (A) Expression of mutants of the metal binding residues of MntA and titratable residues of MntB. (B) Expression of mutants of the hydrophobic seal and the C-terminal truncation (Δ C, as indicated).



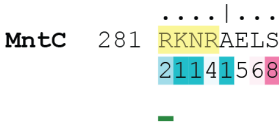
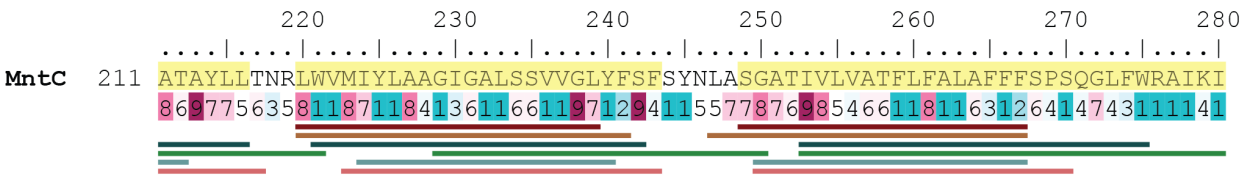
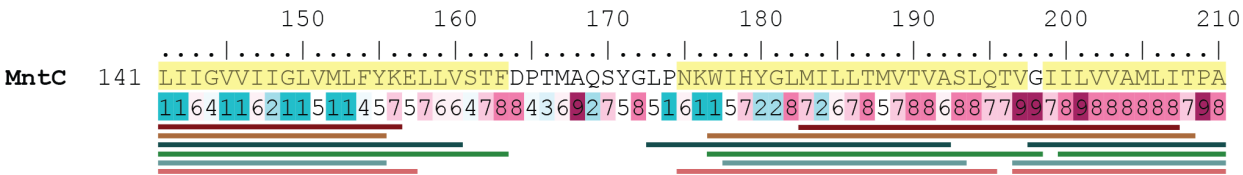
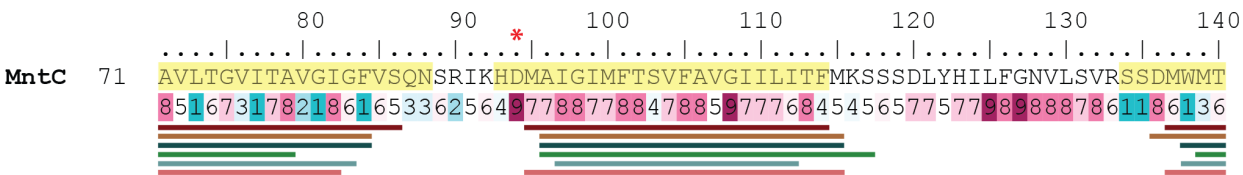
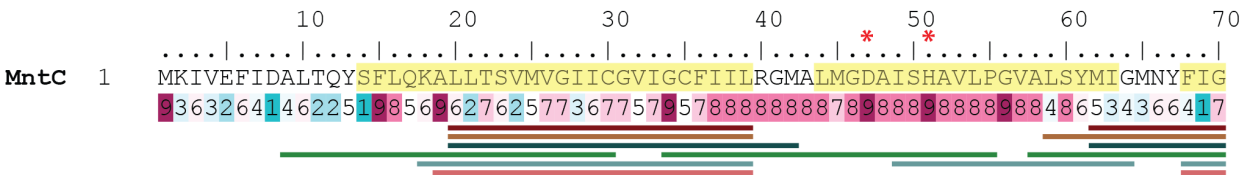
Supplementary Figure 3. Cobalt and nickel do not inhibit baMntBC-A-mediated transport of manganese. (A) Cultures of *Bacillus subtilis* $\Delta mntR$ cells transformed with a control plasmid (open symbols) or a plasmid harboring the complete baMntBC-A operon (full symbols) were grown in LB media (orange symbols) or LB media supplemented with 10 μ M MnSO₄ (black symbols), 50 μ M CoSO₄ (blue symbols), or their combination (red symbols). (B) Same as 'A', only with NiSO₄. Shown are averages of biological triplicates, error bars (shown unless smaller than the symbols) represent standard deviations of the mean.



Supplementary Figure 4. Homology models of baMntBC and their evolutionary conservation. 3-D models of baMntBC were generated by homology modelling using three different templates (PDB IDs 2NQ2 (A), 4G1U (B) and 4R9U (C)). The models are shown as a cartoon representation (upper panel) and colored according to evolutionary conservation. Cyan represents the most variable positions and maroon the most conserved. The lower panel shows the distribution of the most variable positions compared to the most conserved ones, for each model, in spheres representation. Reassuringly, the models are consistent with the expected evolutionary conservation pattern, where the protein's core is composed almost exclusively of conserved residues, while its periphery is enriched with variable residues.



Supplementary Figure 5. Template-independent modelling of baMntBC. Template independent model of (monomeric) baMntC produced using trRosetta. (A) Cartoon representation of trRosetta's model (in rainbow representation) superimposed to the 4G1U-based homology model (in gray). The RMSD between the two models is 4.59 angstrom, with the main difference being the position of TM-4 relative to the other helices. (B) Cartoon representation of trRosetta's model (yellow) and the 4G1U-based homology model (gray) with the charged triad and the hydrophobic ladder shown in sticks representation and colored red and blue respectively. (C) Evolutionary conservation pattern of trRosetta's baMntC model shown in cartoon representation (upper panel) and spheres representation (lower panel) colored according to evolutionary conservation as in Supplementary Figure 4.



- HMMTOP
- PHOBIUS
- TMHMM
- TOPGRAPH
- MEMSAT-SVM
- TOPCONS

Conservation

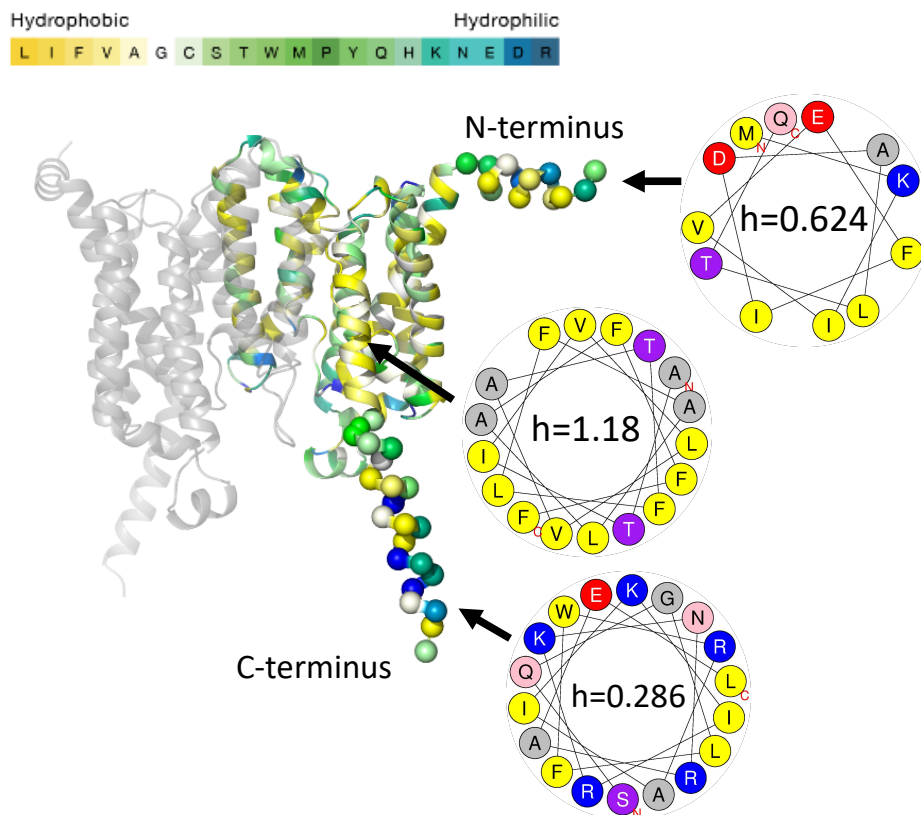
1 2 3 4 5 6 7 8 9

Variable Average Conserved

RAPTORX (ab initio modelling)

—

Supplementary Figure 6. Conserved residues of baMntBC and assignment of transmembrane helices for baMntC. The amino acids sequence of baMntC annotated with the putative transmembrane helices predicted by different computational tools. Namely, HMMTOP, PHOBIUS, TMHMM, TOPGRAPH, MEMSAT-SVM, TOPCONS and RAPTORX. For each amino acid in the sequence, its conservation grade, based on ConSurf calculations, is shown as numbers (1-to-9) with corresponding colors (cyan-through-maroon). Cyan (1) represents the most variable positions and maroon (9) the most conserved. The titratable residues that are suggested by the models to line the translocation cavity (D47, H51 and D94) are marked with red asterisks.



Supplementary Figure 7. Hydrophobicity analysis of baMntC. (A) baMntC model structure in cartoon representation with one monomer colored according to Kesesi & Ben-Tal's hydrophobicity scale. The N- and C-termini are shown in spheres representation. (B) Helical wheel representation and Roseman hydrophobicity scale of the N- and C-termini and of a representative TM helix (helix 9), as indicated. As shown, the N-terminus helix is amphipathic with intermediate hydrophobicity, the TM helix is highly hydrophobic and not amphipathic, and the C-terminus is highly hydrophilic and not amphipathic.

Supplementary Table 1. RMSD between the four baMntBC models.

	trRosetta's model	2NQ2 based model	4G1U based model
2NQ2 based model	5.6 Å		
4G1U based model	4.83 Å	3.5 Å	
4R9U based model	5.48 Å	2.9 Å	4.36 Å